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(54) Title: CREATINE ESTER PRONUTRIENT COMPOUNDS AND FORMULATIONS

(57) Abstract: The present invention describes a method for providing creatine to an animal which includes receiving a creatine ester by the animal. The creatine ester is suitable for being modified by the animal to form creatine.

CREATINE ESTER PRONUTRIENT COMPOUNDS AND FORMULATIONS

CROSS REFERENCE TO RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. § 119(e) to United States Provisional Application 60/232,969 filed September 14, 2000, which is herein
5 incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention generally relates to the field of creatine, and particularly
10 to creatine ester pronutrient compounds and formulations.

BACKGROUND OF THE INVENTION

Creatine is an endogenous nutrient produced naturally by the liver in most vertebrates. The uses of creatine are many, including use as a supplement to increase
15 muscle mass and enhance muscle performance as well as in emerging applications in the treatment of neuromuscular disorders.

Typically, creatine is taken up into muscle cells by specific receptors and converted to phosphocreatine by creatine kinase. Muscle cells, including skeletal muscle and the heart muscle, function by utilizing cellular energy released from the
20 conversion of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). The amount of phosphocreatine in the muscle cell determines the amount of time it will take for the muscle to recover from activity and regenerate adenosine triphosphate (ATP). Phosphocreatine is a rapidly accessible source of phosphate required for regeneration of adenosine triphosphate (ATP) and sustained use of the muscle.

25 For example, energy used to expand and contract muscles is supplied from adenosine triphosphate (ATP). Adenosine triphosphate (ATP) is metabolized in the muscle by cleaving a phosphate radical to release energy needed to contract the muscle. Adenosine diphosphate (ADP) is formed as a byproduct of this metabolism. The most common sources of adenosine triphosphate (ATP) are from glycogen and

creatine phosphate. Creatine phosphate is favored as a ready source of phosphate because it is able to resynthesize adenosine triphosphate (ATP) at a greater rate than is typically achieved utilizing glycogen. Therefore, increasing the amount of creatine in the muscle increases the muscle stores of phosphocreatine and has been proven to increase muscle performance and increase muscle mass.

However, creatine itself is poorly soluble in an aqueous solution. Further, creatine is not well absorbed from the gastrointestinal (GI) tract, which has been estimated to have a 1 to 14 percent absorption rate. Thus, current products require large amounts of creatine to be administered to be effective, typically 5 grams or more. Additionally, side effects such as bloating, gastrointestinal (GI) distress, diarrhea, and the like are encountered with these high dosages.

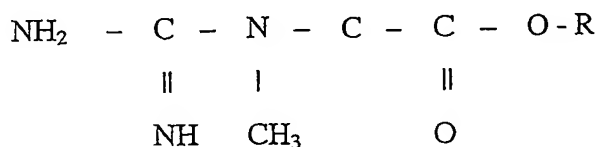
Therefore, it would be desirable to provide an improved approach for enhancing absorption of creatine.

15 SUMMARY OF THE INVENTION

Accordingly, the present invention is directed to creatine ester pronutrients and formulations. In a first aspect of the present invention, a method for providing creatine to an animal includes receiving a creatine ester by the animal. The creatine ester is suitable for being modified by the animal to form creatine.

20 In a second aspect of the present invention, a food supplement includes a creatine ester suitable for being modified by an animal to form creatine. In a third aspect of the present invention, a method for providing creatine to an animal includes receiving an ester derivative of creatine by the animal. The ester derivative of creatine is suitable for acting as a pronutrient in an animal.

25 In a fourth aspect of the present invention, a composition of matter includes:



wherein R represents an ester.

In a fifth aspect of the present invention, a method of producing a creatine pronutrient includes reacting a hydrated form of creatine with an alcohol in an acidic environment wherein a product is formed including a creatine ester pronutrient.

It is to be understood that both the forgoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention as claimed. The accompanying drawings, which are incorporated in and constitute a part of the specification, illustrate an embodiment of the invention and together with the general description, serve to explain the principles of the invention.

10

BRIEF DESCRIPTION OF THE DRAWINGS

The numerous advantages of the present invention may be better understood by those skilled in the art by reference to the accompanying figures in which:

FIG. 1A is an illustration depicting conversion of creatine to creatinine;

15 FIG. 1B is a depiction of an exemplary embodiment of the present invention wherein the processing of creatine monohydrate versus a creatine ester by the body is shown;

FIG. 1C is a flow diagram illustrating an exemplary embodiment of the present invention wherein a pronutrient derivative of creatine is created through the modification of an acid moiety by ester bond attachment;

20 FIG. 1D is an illustration of an embodiment of the present invention in which a graph depicting solubility and partition coefficients of creatine ethyl ester versus creatine monohydrate are shown;

FIGS. 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 2L, 2M and 2N are
25 illustrations of exemplary compounds of the present invention;

FIG. 3 is an illustration depicting an exemplary embodiment of the present invention wherein a creatine ethyl ester compound is produced by solvating creatine monohydrate in dry ethyl alcohol in an acidic atmosphere;

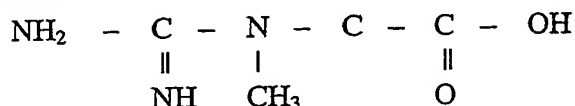
FIG. 4 is an illustration of an embodiment of the present invention wherein additional methods and processes are shown for the production of a creatine ester; and

FIG 5 is an illustration depicting an exemplary embodiment of the present invention wherein a creatine benzyl ester compound is produced by solvating
5 anhydrous creatine in dry benzyl alcohol in an acidic atmosphere.

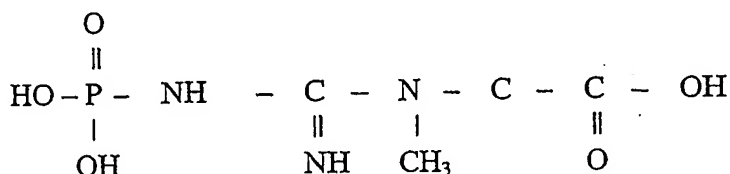
DETAILED DESCRIPTION OF THE INVENTION

Reference will now be made in detail to the presently preferred embodiments
10 of the invention, examples of which are illustrated in the accompanying drawings.

Referring generally now to FIGS. 1 through 5, exemplary embodiments of the present invention are shown. Creatine, N-aminoiminomethyl-N-methylglycine, is an endogenous nutrient which may be produced in the liver and kidneys. Typically, creatine is produced by the transfer of the guanidine moiety of arginine to glycine,
15 which is then methylated to give creatine. Creatine may be represented by the following formula:



20 Creatine phosphate is formed in the body and may be represented by the following formula:



30 Creatine is converted to creatine phosphate by the creatine kinase enzyme. The creatine phosphate transfers its phosphate to adenosine diphosphate (ADP) to accomplish the regeneration of adenosine triphosphate (ATP). Adenosine triphosphate (ATP) may then be utilized by the muscles as a source of energy. Thus,

by providing a formulation and method for enhanced absorption of creatine, the muscle levels of phosphocreatine will be elevated. As a result of this, muscle mass and performance may be increased, thereby permitting a variety of therapeutic applications.

5 Studies in the laboratory have shown that the aqueous solubility and partition coefficient of creatine monohydrate are 15.6 ± 2.1 mg/mL and 0.015 ± 0.007 , respectively. The low oral bioavailability of creatine may derive not only from its low lipophilicity and concomitant poor membrane permeability, but also from rapid conversion to creatinine in the acidic condition of the stomach, and shown in FIG.
10 1A.

At a gastric pH range of 1-2, the equilibrium between creatine and creatinine shifts to the right such that the creatinine/creatine ratio may be greater than or equal to 30. See Edgar, G.; Shiver, H.E., *The Equilibrium Between Creatine and Creatinine in Aqueous Solution. The Effect of Hydrogen Ion*. J. Amer. Chem. Soc. 1925, 47,
15 1179-1188, which is herein incorporated by reference in its entirety.

Referring now to FIG. 1B, an embodiment of the present invention is shown wherein creatine ester metabolism is shown. By providing a creatine ester, a more water-soluble compound will be provided than the relatively insoluble zwitterionic creatine, and increased lipophilicities will allow for better membrane permeability.

20 For example, by masking the carboxylic acid functional group of creatinine by esterification, the formation of creatinine in the stomach will be precluded, resulting in an efficient delivery of the creatine esters to the intestine where absorption may occur. Standard supplements containing creatine monohydrate undergo substantial conversion to creatinine in the stomach. This, coupled with the low absorption of
25 creatine in the intestine, leads to reduced amounts of creatine reaching the muscle cell.

In contrast, creatine esters do not undergo conversion to creatinine in the stomach and are more readily absorbed in the intestine. As a result, blood creatine concentrations are higher and thus more creatine is available to the muscle. As a

result of this, the intestinal absorption of creatine ester will be significantly greater than that observed with creatine monohydrate. An additional advantage of creatine esters is that, as the creatine ester compound moves from the intestinal tissue into the bloodstream, the creatine ester compounds themselves are biologically inactive, but
5 esterase enzymes present in both the intestinal cells and the blood break the ester bonds of creatine ester, converting it to biologically active creatine. In other words, the advantages of the creatine ester are preserved during transport, such as increased solubility and permeability, but when needed, the creatine is available to be converted into its biologically active form.

10 Compared to creatine monohydrate, the increased blood levels of creatine obtained with supplements containing the creatine ester compounds are expected to result in increased responses at the target tissue (i.e. muscle). Thus the increased stability and improved absorption of creatine ester results in much greater blood creatine levels than can be achieved with creatine monohydrate supplements. Once in
15 the blood, creatine is transported into the muscle cells, where it is converted to creatine phosphate that will then be consumed by the cell during muscle performance.

Following is a brief overview of the various disease states that may be responsive to creatine supplementation. It should be noted that the proposed disease states below involve increasing creatine in a diverse array of cells including not only
20 muscle but neurons and endothelial cells as well.

Parkinson's Disease

Parkinson's disease depletes dopamine levels in the brain. Energy impairment may play a role in the loss of dopaminergic neurons. Studies involving rats showed
25 that a diet supplemented with creatine for 2 weeks resulted in only a 10% reduction in brain dopamine as compared to a 70% dopamine depletion in nonsupplemented rodents. See Matthews RT, Ferrante RJ, Klivenyi P, Yang L, Klein AM, Mueller G, Kaddurah-Daouk R and Beal MF. *Creatine and cyclocreatine attenuate MPTP neurotoxicity*. Exp Neurol 157: 142-149, (1999), which is herein incorporated by

reference in its entirety. These pre-clinical studies suggest that creatine dietary supplements may have a positive therapeutic outcome in slowing the onset and decreasing the severity of the disease.

5 Huntington's Disease

Alterations in energy production may also contribute to the development of brain lesions in patients with Huntington's disease. Rats fed a diet supplemented with creatine for 2 weeks responded better when exposed to 3-nitropropionic acid which mimics the changes in energy metabolism seen with Huntington's disease. The
10 creatine fed animals had 83% less lesion volume than nonsupplemented animals (Matthews et al., 1999).

Mitochondrial Pathologies

Creatine supplementation increased the life-span of GP3A transgenic mice (a
15 model for amyotrophic lateral sclerosis) up to 26 days. A study involving patients with a variety of neuromuscular disorders also benefited from creatine supplementation. See Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA, Mueller G, Wermer M, Kaddurah-Daouk R and Beal MF. *Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis*. Nat Med 5: 347-350, (1999), which is herein incorporated by
20 reference in its entirety. Increases in high-density strength measurements were seen in these patients following a short-term trail of creatine (10g/d for 5 days with 5g/d for 5 to 7 days). Creatine supplementation also resulted in increased body weight in these patients.

25

Stroke

Creatine may also be useful in patients with hypoxia and ischemic brain diseases such as stroke. Creatine has been shown to reduce damage to the brainstem and hippocampus resulting from hypoxia. See Balestrino M, Rebaudo R and Lunardi
30 G. *Exogenous creatine delays anoxic depolarization and protects from hypoxic*

damage: *Dose-effect relationship*. Brain Res 816:124-130, (1999); and Dechent P, Pouwels PJ, Wilken B, Hanefeld F and Frahm J. *Increase of total creatine in human brain after oral supplementation of creatine-monohydrate*. Am J Physiol 277: R698-R704, (1999) which are herein incorporated by reference in their entirety. This
5 neuroprotection may be due to prevention of ATP depletion. Studies suggest that supplementation of humans with creatine does increase brain levels of creatine. See Wick M, Fujimori H, Michaelis T and Frahm J. *Brain water diffusion in normal and creatine-supplemented rats during transient global ischemia*. Magn Reson Med 42: 798-802, (1999); Michaelis T, Wick M, Fujimori H, Matsumura A and Frahm J.
10 *Proton MRS of oral creatine supplementation in rats. Cerebral metabolite concentrations and ischemic challenge*. NMR Biomed 12: 309-314, (1999); and Malcon C, Kaddurah-Daouk R and Beal M. *Neuroprotective effects of creatine administration against NMDA and malonate toxicity*. Brain Res 860: 195-198, (2000) which are herein incorporated by reference in their entirety. High brain creatine
15 levels may offer protection to ischemic brain injury.

Muscular Diseases

Patients with various muscular dystrophies supplemented with creatine for 8 weeks showed a 3% increase in strength and a 10% improvement in neuromuscular
20 symptom score. Short-term creatine supplementation also improved strength in patients with rheumatoid arthritis, but did not change physical function. See Felber S, Skladal D, Wyss M, Kremser C, Koller A and Sperl W. *Oral creatine supplementation in Duchenne muscular dystrophy: A clinical and ³¹P magnetic resonance spectroscopy study*. Neurol Res 22: 145-150 (2000), which is herein
25 incorporated by reference in its entirety. Patients with McArdles disease showed improvements when given creatine. The improvements included reduced frequency of muscle pain and increased exercise performance and strength. Increases in exercise performance were also seen during ischemic episodes. See Willer B, Stucki G, Hoppeler H, Bruhlmann P and Krahenbuhl S. *Effects of creatine supplementation*

on muscle weakness in patients with rheumatoid arthritis. *Rheumatology* 39: 293-298, (2000), which is herein incorporated by reference in its entirety.

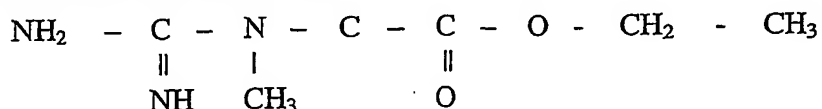
Heart Disease

5 Given the role of creatine phosphate as an immediate and readily accessible source of phosphate for regeneration of ATP, it follows that creatine supplementation may have a favorable impact diseases of the heart. In patients with congestive heart failure creatine supplementation produced an increase in exercise performance as measured by strength and endurance. See Gordon A, Hultman E, Kaijser L, 10 Kristjansson S, Rolf CJ, Nyquist O and Sylven C. *Creatine supplementation in chronic heart failure increases skeletal muscle creatine phosphat and muscle performanmce*. *Cardiovasc Res* 30: 413-418, (1995), which is herein incorporated by reference in its entirety. An additional consideration with ramifications in the management of cardiovascular diseases is the report that creatine supplementation can 15 lower cholesterol and triglyceride levels in humans. See Earnest CP, Almada AL and Mitchell TL. *High-performance capillary electrophoresis-pure creatine monohydrate reduces blood lipids in men and women*. *Clin Sci (Colch)* 91: 113-118, (1996), which is herein incorporated by reference in its entirety.

20 Muscle Fatigue Secondary to Aging

Research on adults over 60-years of age suggest that creatine supplementation may delay muscle fatigue, but does not affect body composition or strength (Rawson and Clarkson, 2000). See Rawson ES and Clarkson PM. *Acute creatine supplementation in older men*. *Int J Sports Med* 21: 71-75, (2000), which is herein 25 incorporated by reference in its entirety. As with many of the therapeutic implication studies, these preliminary experiments were performed over a short (i.e. less than 30-day) period of time, where the effects of creatine supplementation on muscle mass and strength may not be fully demonstrated. While the effects observed in the elderly were not profound, these initial reports suggest the health benefits to this growing 30 population are promising.

Referring now to FIG. 1C, an exemplary embodiment of the present invention is shown wherein a pronutrient derivative of creatine is created through the modification of an acid moiety by ester bond attachment. Creatine 102 is changed by modifying an acid moiety through ester bond attachment 104. For example, creatine
 5 may be converted to creatine ethyl ester 106, which has a formula as follows:

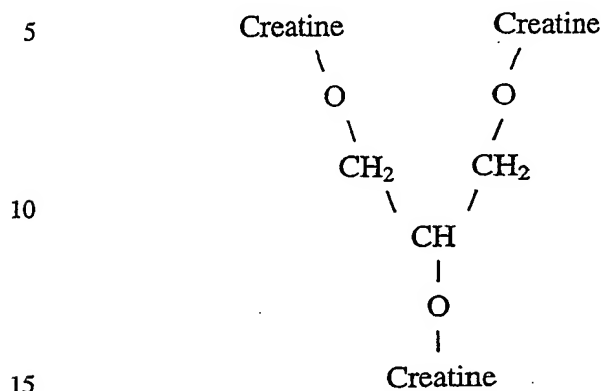


A creatine ester has the advantages of increased aqueous solubility, increased
 10 absorption from the gastrointestinal (GI) tract resulting in increased bioavailability, and increased stability, especially for solution formulations. Increased bioavailability allows smaller doses to be utilized with greater effect, thereby resulting in fewer gastrointestinal side effects. Further, more varied formulation possibilities are feasible, for example, the product may be formulated in tablet or capsule form with
 15 dextrose and/or phosphate for ease of use and effectiveness.

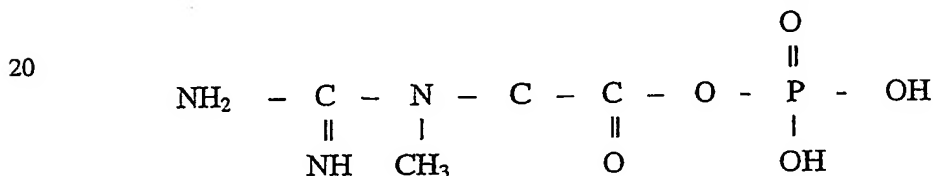
Once the product is ingested 108, the body metabolizes and activates the product by esterases 110, which may be found in the intestinal lumen, epithelial cells and the blood. The esterases convert the product to creatine 114 and an alcohol 116. Thus, the current invention supplements the amount of creatine normally available to
 20 the muscle thereby increasing phosphocreatine levels and decreasing the recovery time required before the muscle can perform activity. Further, the resultant alcohols, such as ethanol, glycerol, benzyl alcohol, tert-butyl alcohol, are relatively harmless. *See* Budavari, S. (Ed.) The Merck Index. Merck and Co., Inc., Whitehouse Station, NJ, 1996, which is herein incorporated by reference in its entirety. For example,
 25 benzyl alcohol is used as a pharmaceutical preservative.

Solubility and permeability are two important factors in the amount of a compound made available to an organism, otherwise known as bioavailability. Solubility refers to the amount of the compound that may be dissolved, wherein permeability refers to the ability of the compound to penetrate across a barrier, such
 30 as a membrane, cell wall and the like. In terms of solubility, creatine ethyl ester is a

2J, 2K, 2L, 2M and 2N. For example, a mono-creatine glycerol, di-creatine glycerol, triscreatine glycerol and the like, may be utilized as a pronutrient of the present invention, the formula for a triscreatine glycerol is as follows:



Another example of a creatine ester compound suitable for use as a pronutrient includes creatine phosphoester, the formula of which is as follows:



25 Thus, the present invention provides multiple ester derivatives of creatine for use as pronutrients having increased solubility and permeability over creatine itself. The advantages of creatine pronutrients of the present invention would be useful in athletic performance markets, therapeutic markets targeting patients with diseases involving reduced muscle performance/loss of muscle mass, livestock/animal food
30 products market, and the like.

Referring generally now to FIGS. 3 and 4, an exemplary embodiment of the present invention is shown wherein the production of an ester derivative of creatine is shown. A creatine ester may be formed by reacting a hydrated form of creatine or anhydrous creatine with various alcohols in an acidic atmosphere. Under these

great deal more soluble than creatine. Utilizing a physiological buffer solution (PBS), laboratory analysis indicates that creatine monohydrate has a solubility limit of approximately 10 mg/ml. This value may be overly generous, as a great deal of vortexing of the sample and brief heating of the sample to 37 degrees Celsius had to be performed to even achieve that result. However, the creatine ethyl ester is readily soluble in room temperature PBS with solubility over 200 mg/ml.

With regard to permeability, a laboratory analysis was performed comparing the creatine monohydrate to creatine ethyl ester in MDCK monolayers. The MDCK are a canine kidney epithelial cell line that has been used as an in vitro model for assessing drug permeability. In the MDCK monolayers, creatine monohydrate showed approximately 10% flux over one hour. In other words, 10% of the original amount of creatine monohydrate added to one side of the MDCK monolayer made it across to the other side in a 60-minute period. For creatine ethyl ester, the permeability is quite higher, averaging approximately 20% flux over one hour. Similar results are expected in a Caco-2 monolayer, which may be used as an in vitro model for intestinal absorption. Thus, the creatine ester of the present invention has the unexpected result of both increased solubility and membrane permeability, and thus greater bioavailability, as shown through the following table and graph depicted in FIG. 1D.

Substance	Conc. at Saturation mg/ml	Partition Coefficient
Creatine	15.6 +/- 2.1	0.015 +/- 0.007
Creatine Ethyl Ester	205.9 +/- 1.5	0.074 +/- 0.008
Creatine Benzyl Ester	89.26 +/- 0.8	0.106 +/- 0.01

Although a creatine ethyl ester compound has been described, it should be apparent that a wide variety of creatine ester compounds and salts thereof are contemplated by the present invention without departing from the spirit and scope thereof, examples of which are shown in FIGS. 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I,

conditions, various ester procreateine compounds may be formed, generally as white precipitates. The resultant creatine esters may be further purified by solvating in an alcohol at elevated temperatures and then cooling to form the ester procreateine compound. The final recrystallization step may not be required, as the initial
5 precipitate is generally pure. However, such an extra step may be useful to ensure that the purest form of the creatine pronutrient has been obtained.

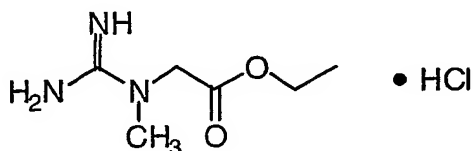
For example, as shown in FIG. 3, creatine monohydrate may be solvated in dry ethyl alcohol in an atmosphere of hydrochloric acid at ambient temperatures. The resultant creatine ethyl ester compound is solid at ambient temperatures. While not
10 functionally necessary, the resultant creatine ethyl ester may be further purified with the use of ethyl alcohol at an elevated temperature to solvate the creatine ethyl ester away from possible contaminants contained in the solid reaction material. Purified creatine ethyl ester may then be achieved upon cooling the solvated creatine ethyl ester. It should also be apparent that anhydrous creatine may also be utilized without
15 departing from the spirit and scope of the present invention.

Although the formulation of creatine ethyl ester is disclosed, it should be apparent that a variety of creatine esters may be produced utilizing analogous reaction systems without departing from the spirit and scope of the present invention. See Dox., A.W.; Yoder, L. *Esterification of Creatine*. J. Biol. Chem. 1922, 67, 671-673,
20 which is herein incorporated by reference in its entirety. For instance, a variety of methods of producing a creatine ester are contemplated without departing from the spirit and scope of the present invention, such as the methods and process shown in FIG. 4, wherein X may include a leaving group. Although the use of creatine monohydrate is disclosed, a variety of creatine containing starting compounds are
25 contemplated by the present invention, creatine monohydrate being disclosed merely because of its availability.

Referring now to FIG. 5, an embodiment of the present invention is shown wherein anhydrous creatine is solvated in dry benzyl alcohol in an atmosphere of hydrochloric acid at ambient temperatures to produce a creatine ester. The resultant

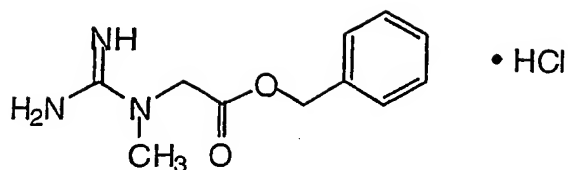
creatine benzyl ester compound is a white solid at ambient temperatures. While not functionally necessary, the resultant creatine benzyl ester may be further purified with the use of ethyl alcohol at an elevated temperature to solvate the creatine benzyl ester away from possible contaminants. Purified creatine benzyl ester may then be achieved upon cooling the solvated creatine benzyl ester. As stated earlier, the final recrystallization step may not be required as the initial precipitate is relatively pure. However, such an extra purification step may be useful to ensure that the most pure form of the compound has been obtained.

As discussed earlier, creatine esters may also be synthesized from anhydrous creatine using esterification methods and isolated as their hydrochloride salts. For example, creatine ethyl ester hydrochloride may be synthesized by treatment of anhydrous creatine with ethanolic HCl at room temperature. *See* Dox., A.W.; Yoder, L. *Esterification of Creatine*. J. Biol. Chem., 67, 671-673, (1922) which is herein incorporated by reference in its entirety.

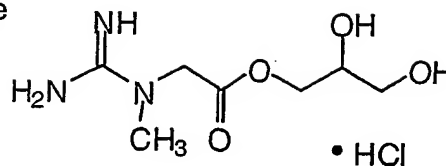


creatine ethyl ester hydrochloride

Using this method, creatine ethyl ester hydrochloride was synthesized in 74% yield after a single recrystallization from ethanol.

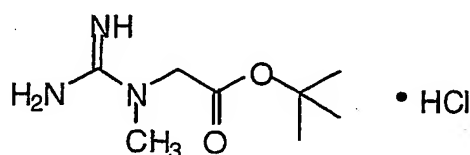


creatine benzyl ester hydrochloride



creatine monoglycerate ester hydrochloride

Creatine esters creatine benzyl ester hydrochloride and creatine monoglycerate ester hydrochloride may similarly be obtained by exposure of
 5 anhydrous creatine with excess HCl-saturated benzyl alcohol and glycerol, respectively. It should be apparent that stereoisomers, such as a stereoisomers of creatine monoglycerate ester hydrochloride, and the compounds shown in FIGS. 2B, 2E, 2F, 2G, 2J and the like, are also contemplated by the present invention.

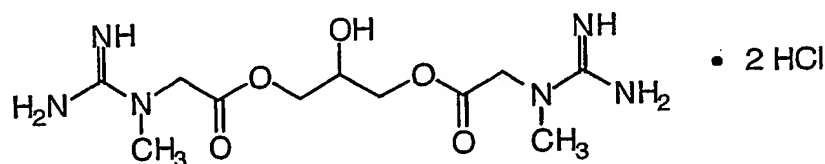


creatine *tert*-butyl ester hydrochloride

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Creatine *tert*-butyl ester hydrochloride may be obtained by treatment of creatine acid chloride with *tert*-butanol and zinc chloride. See Rak, J.; Lubkowski, J.;
 15 Nikel, I.; Przubulski, J.; Blazejowski, J. *Thermal Properties, Crystal Lattice Energy, Mechanism and Energetics of the Thermal Decomposition of Hydrochlorides of 2-Amino Acid Esters*, *Thermochimica Acta* 171, 253-277 (1990); Yadav, J.S.; Reddy, G.S.; Srinivas, D.; Himabindu, K. *Zinc Promoted Mild and Efficient Method for the*

- Esterification of Acid Chlorides with Alcohols*, Synthetic Comm. 28, 2337-2342 (1998). Creatine *tert*-butyl ester hydrochloride may also be obtained by treatment of anhydrous creatine with *tert*-butanol and anhydrous magnesium sulfate and catalytic sulfuric acid. See Wright, S.W.; Hageman, D.L.; Wright, A.S.; McClure, L.D.
- 5 *Convenient Preparations of t-Butyl Esters and Ethers from t-Butanol*, Tetrahedron Lett. 38, 7345-7348 (1997), which are herein incorporated by reference in their entireties.



bis creatine glycerate ester dihydrochloride

- Bis creatine glycerate ester dihydrochloride ester, may be obtained by
- 10 treatment of creatine acid chloride with a half-molar equivalent of anhydrous glycerol. See Rak, J.; Lubkowski, J.; Nikel, I.; Przubulski, J.; Blazejowski, J. *Thermal Properties, Crystal Lattice Energy, Mechanism and Energetics of the Thermal Decomposition of Hydrochlorides of 2-Amino Acid Ester*, *Thermochimica Acta* 71, 253-277 (1990), which is herein incorporated by reference in its entirety.

- 15 Alternatives to these methods include transesterification reaction of CE1 using either catalytic diphenyl ammonium triflate and trimethylsilyl chloride (Wakasugi et al., 2000) or catalytic potassium *tert*-butoxide and 1 equivalent of *tert*-butyl acetate. Creatine acid chloride may also be used rather than anhydrous creatine in the esterification reactions. See Wakasugi, K.; Misake, T.; Yamada, K.; Tanabe, Y.
- 20 *Diphenylammonium triflate (DPAT): Efficient Catalyst for Esterification of Carboxylic Acids and For Transesterification of Carboxylic Esters With Nearly Equimolar Amounts of Alcohols*, Tetrahedron Lett. 41, 5249-5252 (2000), which is herein incorporated by reference in its entirety.

- Regioselectivity problems in the formation of creatine esters, such as creatine
- 25 monoglycerate ester hydrochloride, Bis creatine glycerate ester dihydrochloride ester,

and the like, may be addressed by selective esterification of the primary alcohol functional group(s) of glycerol with creatine acid chloride in the presence of *N,N*-diisopropylethylamine or 2,4,6-collidine at low temperatures. See Ishihara, K.; Kurihara, H.; Yamamoto, H. *An Extremely Simple, Convenient, and Selective Method for Acetylating Primary Alcohols in the Presence of Secondary Alcohols*, J. Org Chem. 58, 3791-3793 (1993), which is herein incorporated by reference in its entirety.

Creatine esters may be purified by crystallization, flash column chromatography, and the like, if desired, and the structures and purity confirmed by analytical HPLC, ^1H and ^{13}C NMR, IR, melting point and elemental analysis. The following data was obtained through nuclear magnetic resonance spectroscopy of the corresponding compounds:

15 Creatine ethyl ester hydrochloride

^1H NMR (500 MHz, CDCl_3) δ 1.12 (dq, $J = 6.0$ Hz, $J = 1.0$ Hz, 3H), 2.91, (s, 3H), 4.10-4.11 (m, 4H).

20 Creatine benzyl ester hydrochloride

^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ 3.03 (s, 3H), 4.13 (s, 2H), 5.06 (s, 2H), 7.22-7.38 (m, 5H).

25 It is understood that the specific order or hierarchy of steps in the methods disclosed are examples of exemplary approaches. Based upon design preferences, it is understood that the specific order or hierarchy of steps in the method can be rearranged while remaining within the scope of the present invention. The accompanying method claims present elements of the various steps in a sample order, and are not meant to be limited to the specific order or hierarchy presented.

30 It is believed that the creatine ester pronutrient compounds and formulations of the present invention and many of its attendant advantages will be understood by

the forgoing description. It is also believed that it will be apparent that various changes may be made in the form, construction and arrangement of the components thereof without departing from the scope and spirit of the invention or without sacrificing all of its material advantages. The form herein before described being
5 merely an explanatory embodiment thereof. It is the intention of the following claims to encompass and include such changes.

CLAIMS

What is claimed is:

- 1 1. A method for providing *creatine* to an animal, comprising:
2 receiving a creatine ester by the animal, wherein the creatine ester is suitable
3 for being modified by the animal to form creatine.
1
- 1 2. The method as described in claim 1, wherein the creatine ester is suitable for
2 being formed in a solid form capable of being ingested by the animal.
1
- 1 3. The method as described in claim 2, wherein the solid form includes the
2 creatine ester and at least one of dextrose and phosphate.
1
- 1 4. The method as described in claim 2, wherein the solid form is configured as at
2 least one of a tablet and a capsule.
1
- 1 5. The method as described in claim 1, wherein the creatine ester is suitable for
2 liquid delivery.
1
- 1 6. The method as described in claim 5, wherein the creatine ester includes at
2 least one of an aqueous solution and emulsion.
1
- 1 7. The method as described in claim 1, wherein the creatine ester includes at
2 least one of creatine ethyl ester, creatine benzyl ester, creatine phosphoester,
3 mon creatine glycerol, t-butyl creatine ester, dicreatine glycerol and
4 tricreatine glycerol.
1
- 1 8. The method as described in claim 1, wherein the creatine ester is received by
2 the animal, the creatine ester is modified by the animal into creatine and an

3 alcohol.

1

1 9. The method as described in claim 8, wherein the creatine ester is modified by
2 the animal into creatine and alcohol by an esterase.

1

1 10. The method as described in claim 8, wherein the creatine ester is modified by
2 at least one of an intestinal lumen, epithelial cell and blood of the animal into
3 creatine.

1

1 11. The method as described in claim 1, further comprising forming a creatine
2 ester, wherein an acid moiety of creatine is modified to provide an ester bond.

1

1 12. The method as described in claim 1, wherein the animal includes a human and
2 livestock.

1

1

- 1 13. A food supplement, comprising:
2 a creatine ester suitable for being modified by an animal to form creatine.
1
- 1 14. The food supplement as described in claim 13, wherein the creatine ester is
2 suitable for being formed in a solid form capable of being ingested by the
3 animal.
1
- 1 15. The food supplement as described in claim 14, wherein the solid form
2 includes the creatine ester and at least one of dextrose and phosphate.
1
- 1 16. The food supplement as described in claim 14, wherein the solid form is
2 configured as at least one of a tablet and a capsule.
1
- 1 17. The food supplement as described in claim 13, wherein the creatine ester is
2 suitable for liquid delivery.
1
- 1 18. The food supplement as described in claim 17, wherein the creatine ester
2 includes at least one of an aqueous solution and emulsion.
1
- 1 19. The food supplement as described in claim 13, wherein the creatine ester
2 includes at least one of creatine ethyl ester, creatine benzyl ester, creatine
3 phosphoester, mon creatine glycerol, t-butyl creatine ester, dicreatine glycerol
4 and tricreatine glycerol.
1
- 1 20. The food supplement as described in claim 13, wherein the creatine ester is
2 received by the animal, the creatine ester is modified by the animal into
3 creatine and an alcohol.
1
- 1 21. The food supplement as described in claim 20, wherein the creatine ester is

2 modified by the animal into creatine and alcohol by an esterase.

1

1 22. The food supplement as described in claim 20, wherein the creatine ester is
2 modified by at least one of an intestinal lumen, epithelial cell and blood of the
3 animal into creatine.

1

1 23. The food supplement as described in claim 13, further comprising forming a
2 creatine ester, wherein an acid moiety of creatine is modified to provide an
3 ester bond.

1

1 24. The food supplement as described in claim 13, wherein the animal includes at
2 least one of human and livestock.

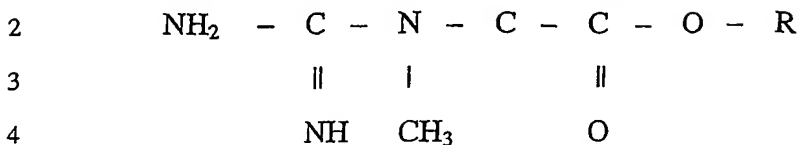
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1

- 1 25. A method for providing creatine to an animal, comprising:
2 receiving an ester derivative of creatine by the animal, wherein the ester
3 derivative of creatine is suitable for acting as a pronutrient in an
4 animal.
- 1
1 26. The method as described in claim 25, wherein the ester derivative of creatine
2 acts as a pronutrient in the gastrointestinal tract of the animal.
- 1
1 27. The method as described in claim 25, wherein the pronutrient is metabolized
2 by the animal to form creatine.
- 1
1 28. The method as described in claim 27, wherein the pronutrient is metabolized
2 by an esterase.
- 1
1 29. The method as described in claim 28, wherein the pronutrient is metabolized
2 by esterases in at least one of an intestinal lumen, epithelial cell and blood.
- 1
1 30. The method as described in claim 25, wherein the pronutrient is metabolized
2 by the animal for form an alcohol.
- 1
1 31. The method as described in claim 25, wherein the creatine ester is suitable for
2 being formed in a solid form capable of being ingested by the animal.
- 1
1 32. The method as described in claim 31, wherein the solid form includes the
2 creatine ester and at least one of dextrose and phosphate.
- 1
1 33. The method as described in claim 31, wherein the solid form is configured as
2 at least one of a tablet and a capsule.
- 1

- 1 34. The method as described in claim 25, wherein the creatine ester is suitable for
2 liquid delivery.
1
- 1 35. The method as described in claim 34, wherein the creatine ester includes at
2 least one of an aqueous solution and emulsion.
1
- 1 36. The method as described in claim 25, wherein the creatine ester includes at
2 least one of creatine ethyl ester, creatine benzyl ester, creatine phosphoester,
3 mon creatine glycerol, t-butyl creatine ester, dicreatine glycerol and
4 tricreatine glycerol.
1
- 1 37. The method as described in claim 25, wherein the creatine ester is received by
2 the animal, the creatine ester is modified by the animal into creatine and an
3 alcohol.
1
- 1 38. The method as described in claim 37, wherein the creatine ester is modified by
2 the animal into creatine and alcohol by an esterase.
1
- 1 39. The method as described in claim 37, wherein the creatine ester is modified by
2 at least one of an intestinal lumen, epithelial cell and blood of the animal into
3 creatine.
1
- 1 40. The method as described in claim 25, further comprising forming a creatine
2 ester, wherein an acid moiety of creatine is modified to provide an ester bond.
1
- 1 41. The method as described in claim 25, wherein the animal includes human and
2 livestock.
1
1

1 42. A composition of matter, comprising:



5 wherein R represents an ester.

1
1 43. The composition of matter as described in claim 42, wherein R represents an
2 ester so as to form a composition of matter including at least one of creatine
3 benzyl ester, creatine phosphoester, mon creatine glycerol, t-butyl creatine
4 ester, dicreatine glycerol and tricreatine glycerol.

1
1 44. The composition of matter as described in claim 42, wherein the composition
2 of matter is suitable for being converted to creatine upon receipt by an animal.

1
1 45. The composition of matter as described in claim 44, wherein upon receipt of
2 the composition of matter by the animal, creatine and an alcohol are formed.

1
1 46. The composition of matter as described in claim 42, wherein a salt is formed
2 of the composition of matter.

- 1 47. A method of producing a creatine pronutrient, comprising:
2 reacting at least one of an anhydrous creatine and hydrated form of creatine
3 with an alcohol in an acidic environment, wherein a product is formed
4 including a creatine ester pronutrient.
1
- 1 48. The method as described in claim 47, wherein the hydrated form of creatine
2 includes creatine monohydrate.
1
- 1 49. The method as described in claim 47, wherein the alcohol includes at least one
2 of ethyl alcohol and benzyl alcohol.
1
- 1 50. The method as described in claim 47, wherein the acidic environment is
2 achieved through hydrochloric acid being present.
1
- 1 51. The method as described in claim 47, further comprising purifying the
2 product.
1
- 1 52. The method as described in claim 51, wherein the product is purified by
2 solvating the product in an alcohol and then cooling to form purified creatine
3 ester.
1
1

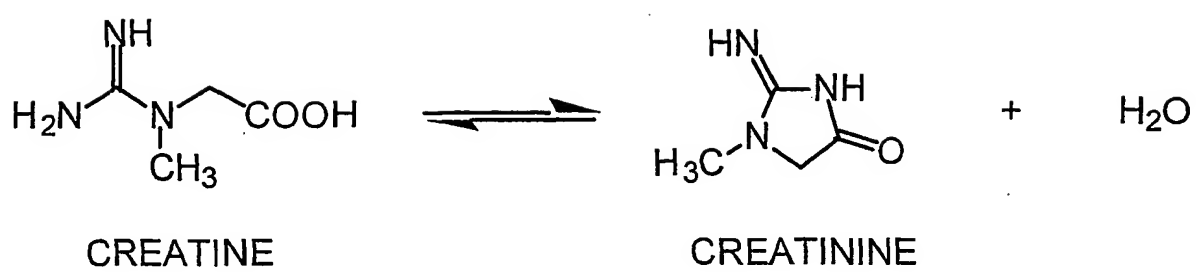


FIG. 1A

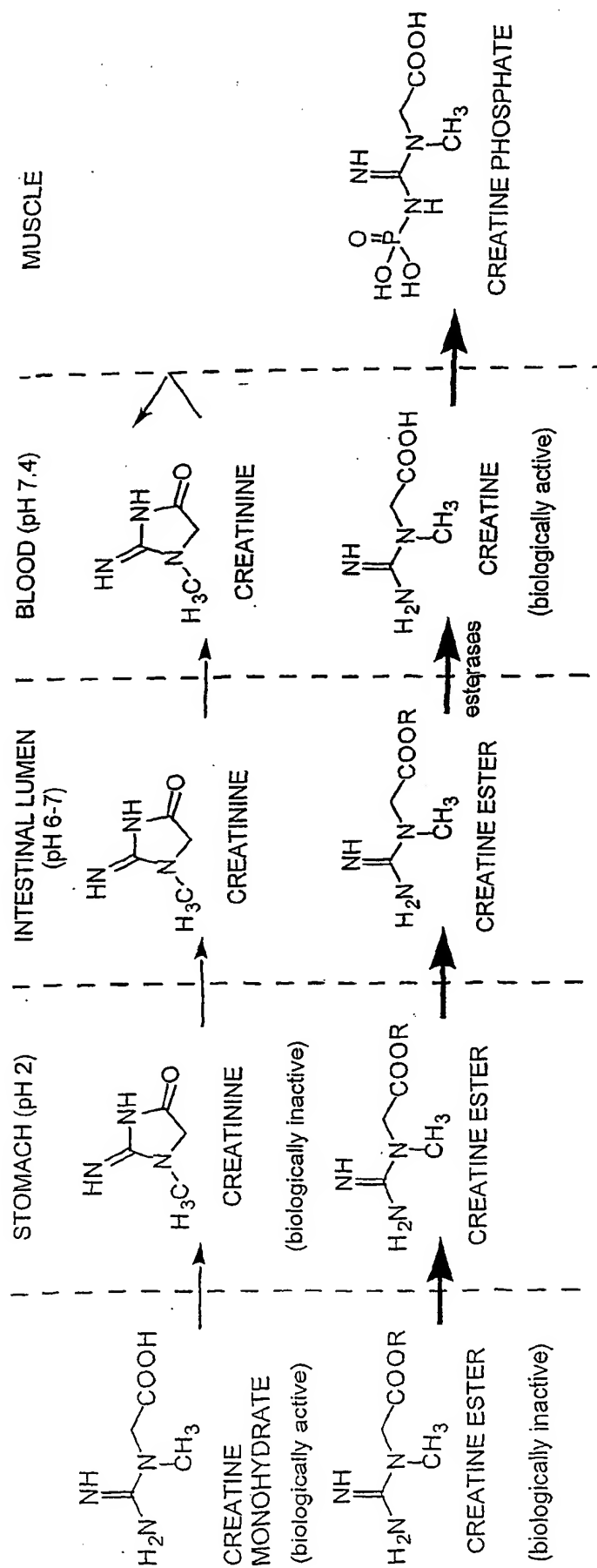


FIG. 1B

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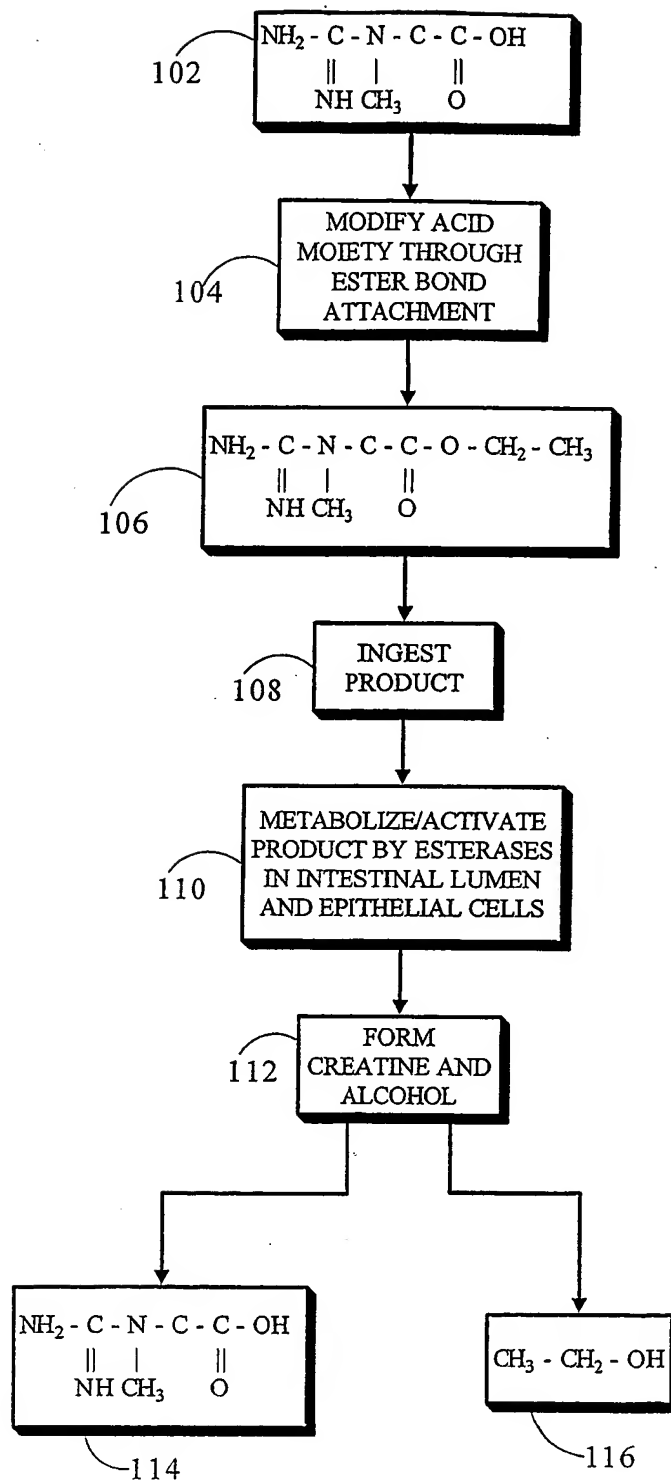


FIG. 1C

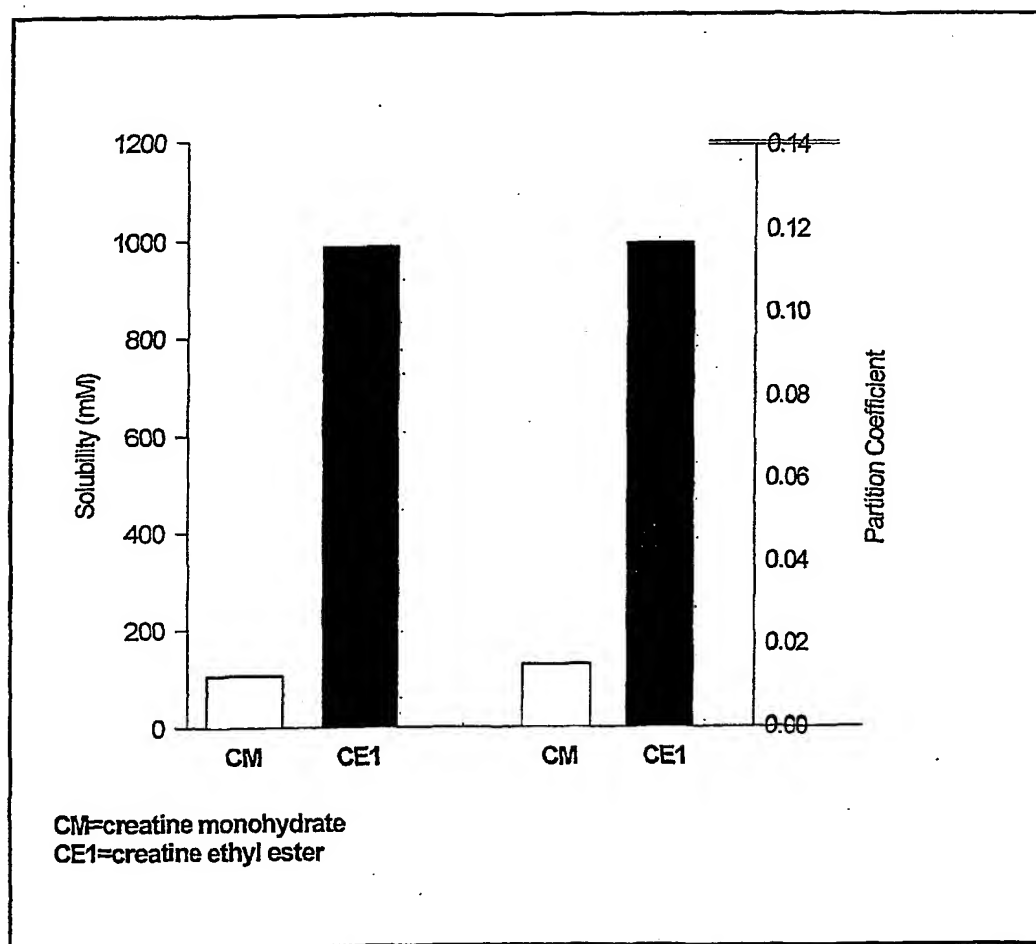
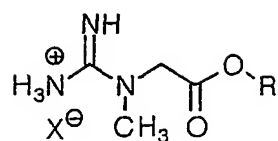


FIG. 1D



R = Et, Benzyl, and the like

X = Stearate, Palmitate, Oleate, Lauryl Sulfate, Chloride, Acetate, Succinate, Mesylate, Sulfate, Citrate, and the like

FIG. 2A

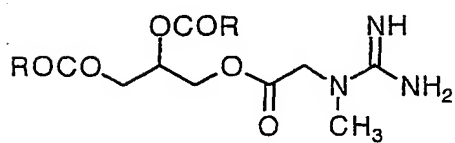


FIG. 2B

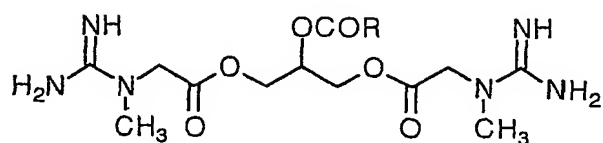
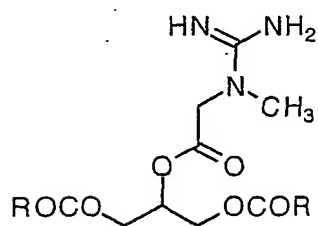


FIG. 2C



R = Ethyl, Benzyl, and the like

FIG. 2D

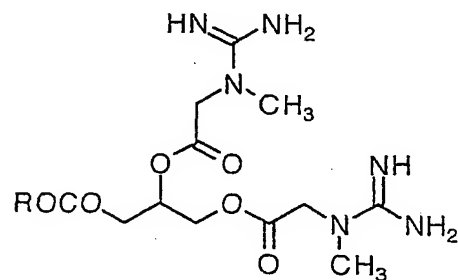


FIG. 2E

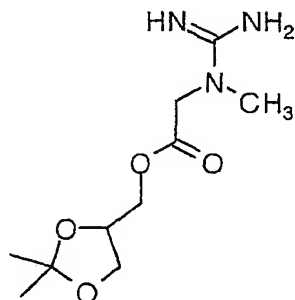


FIG. 2F

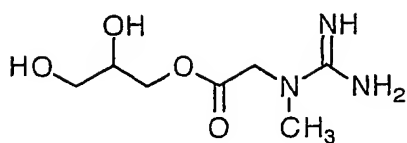


FIG. 2G

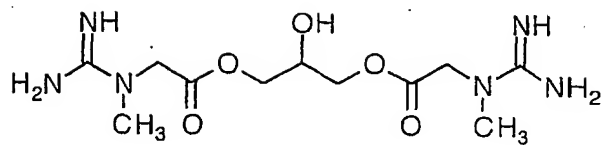


FIG. 2H

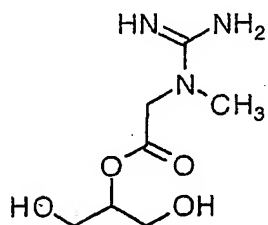


FIG. 2I

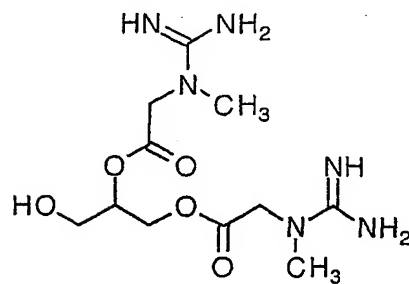


FIG. 2J

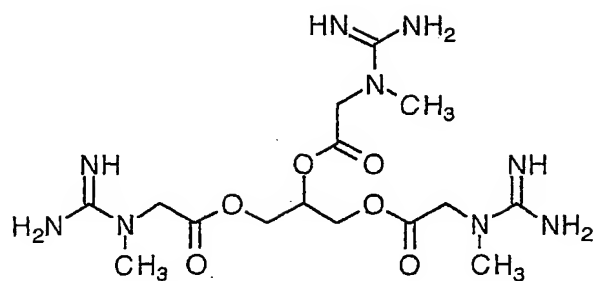


FIG. 2K

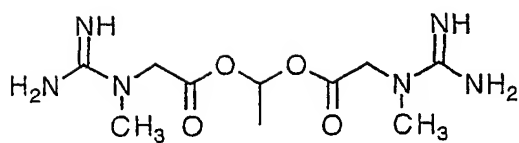


FIG. 2L

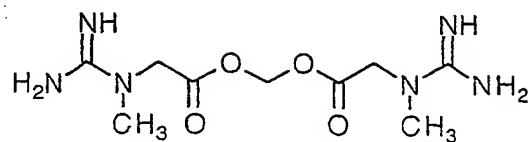


FIG. 2M

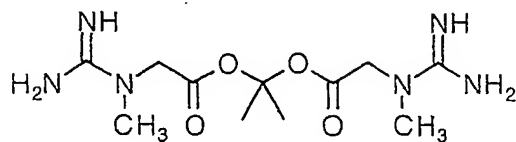


FIG. 2N

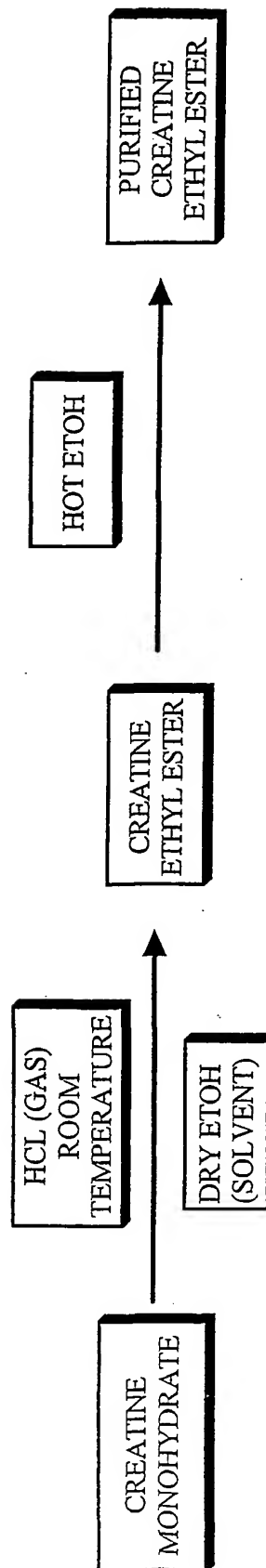


FIG. 3

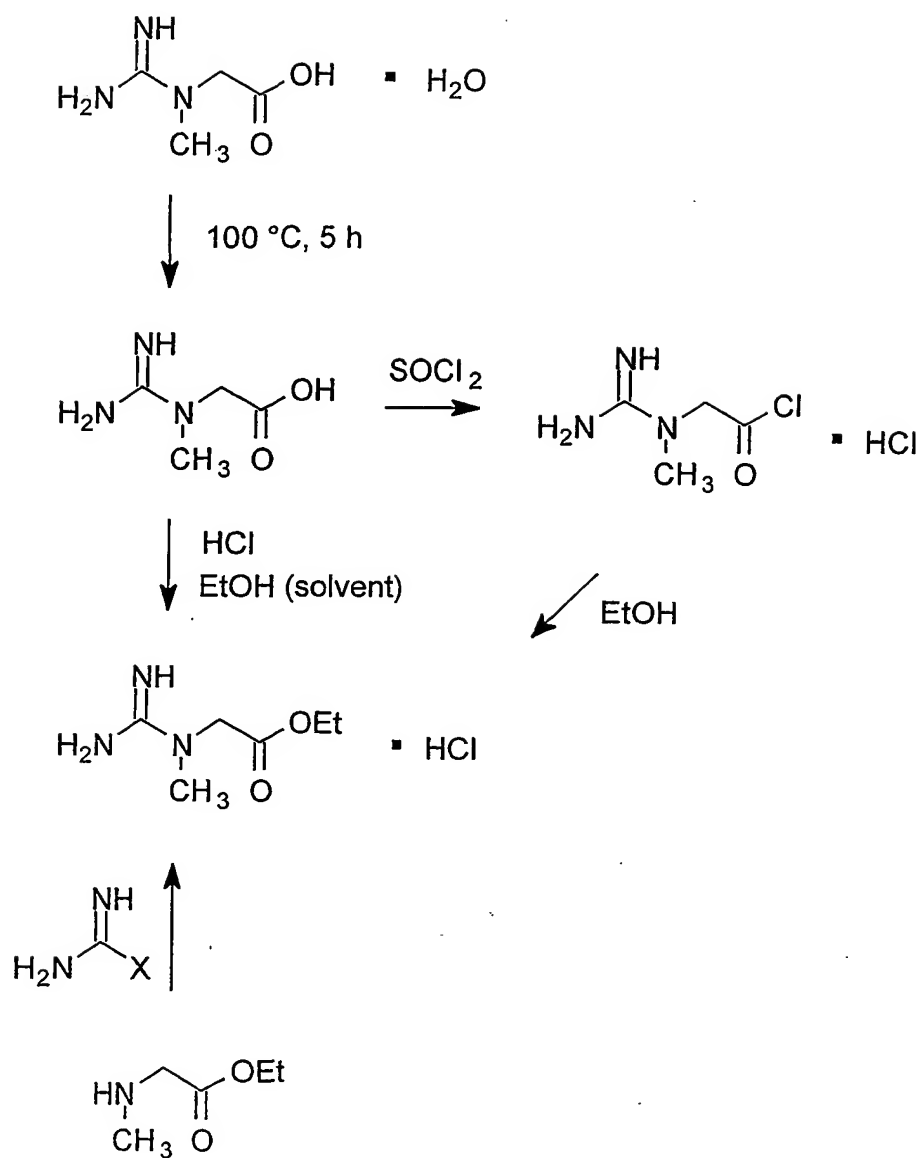


FIG. 4

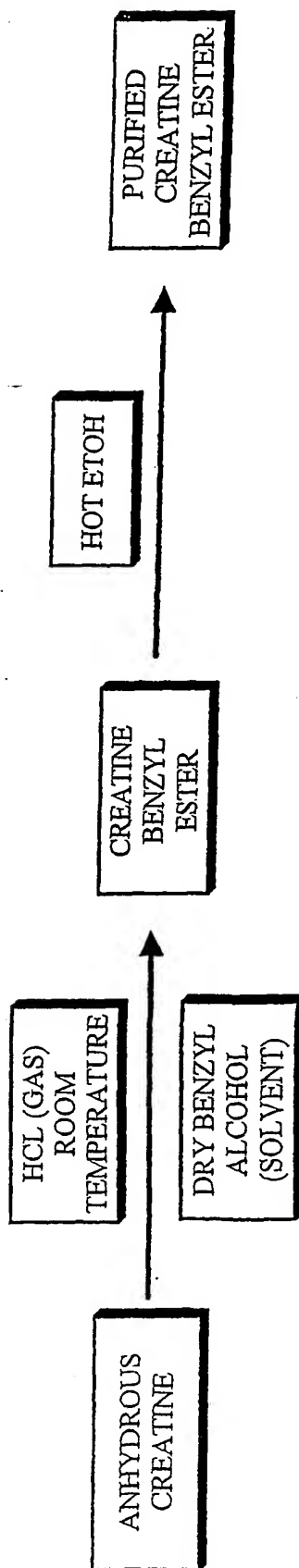


FIG. 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28788

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 31/661, 31/221, 9/48, 9/20

US CL :514/75, 551; 424/439, 451, 464

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/75, 551; 424/439, 451, 464

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, USPATFULL, EPO, JPO, DERWENT, STN, BIOSCIENCE, REGISTRY

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 6,136,339 A (GARDINER) 24 October 2000 (24.10.00), see entire document.	1-52
Y	US 5,576,316 A (COHN) 19 November 1996 (19.11.96), see abstract, column 2, lines 58-67, column 3, lines 64-66, column 4, lines 23-65, column 5, lines 35-43.	1-52
A	US 5,994,581 A (FANG) 30 November 1999 (30.11.99) see abstract, and entire document.	1-52
A	US 6,117,872 A (MAXWELL et al.) 12 September 2000 (12.09.00) see entire document.	1-52
A	US 6,093,848 A (GREINDL et al.) 25 July 2000 (25.07.00) see entire document.	1-52

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Z" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

02 JANUARY 2002

Date of mailing of the international search report

11 JAN 2002

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28788

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,773,473 A (GREEN et al.) 30 June 1998 (30.06.98) see entire document.	